

### **Amendments to the claims**

This listing of claims will replace all prior versions, and listings, of claims in the application.

#### **Listing of Claims:**

Claim 1 (Currently amended): A method for determining candidates for gene deletions and additions using a model of a metabolic network associated with an organism, the model comprising a plurality of metabolic reactions defining metabolite relationships, the method comprising:

selecting [[a]]at least one bioengineering objective function for the organism;  
selecting at least one cellular objective function;  
forming [[an]]a linear optimization problem that couples the at least one cellular objective function with the at least one bioengineering objective function; and  
solving the linear optimization problem to yield at least one candidate.

Claim 2 (Previously presented): The method of claim 1 further comprising using the at least one candidate to genetically modify the organism.

Claim 3 (Currently amended): The method of claim 1 wherein the bioengineering objective function is overproduction of a chemical.

Claim 4 (Currently amended): The method of claim 1 wherein the bioengineering objective function is underproduction of a chemical.

Claim 5 (Currently amended): The method of claim 1 wherein the cellular objective function is growth.

Claim 6 (Withdrawn): The method of claim 1 wherein the cellular objective is minimization of metabolic adjustment.

Claim 7 (Original): The method of claim 1 wherein the candidate is a candidate for gene deletion, and the optimization problem includes a binary value for specifying if reaction flux is active or inactive.

Claim 8 (Original): The method of claim 1 wherein the optimization problem is a bilevel optimization problem.

Claim 9 (Withdrawn): The method of claim 1 wherein the optimization problem is a mixed-integer optimization problem.

Claim 10 (Original): The method of claim 1 wherein the optimization problem includes at least one stoichiometric constraint.

Claim 11 (Original): The method of claim 1 wherein the optimization problem includes at least one chemical uptake constraint.

Claim 12 (Currently amended): The method of claim 1 wherein the step of forming an optimization problem includes quantifying the cellular objective function as an aggregate reaction flux.

Claim 13 (Currently amended): The method of claim 1 further comprising evaluating performance limits of the metabolic network with the at least one candidate based on ability of the network to meet the at least one cellular objective function.

Claim 14 (Currently amended): The method of claim 1 wherein the cellular objective function is selected from the group consisting of: maximizing a growth rate, maximizing ATP production, minimizing metabolic adjustment, minimizing nutrient uptake, minimizing redox production, minimizing a Euclidean norm, and combinations thereof.

Claim 15 (Withdrawn): The method of claim 1 wherein the bioengineering objective is

overproduction of glycerol and at least one candidate is for gene deletion and comprising genes coding for the enzymes fructose- 1,6-bisphosphatase, fructose- 1,6-bisphosphatase aldolase, phosphoglycerate kinase, glyceraldehydes-3-phosphate dehydrogenase, phosphoenolpyruvate synthase, NADH dehydrogenase I, phosphogluconate dehydratase, 2-keto-3-deoxy-6-phosphofluconate aldolase, triosphosphate isomerase, glucose 6-phosphate-1 -dehydrogenase, 6-phosphogluconolactonase- , deoxyribose-phosphate aldolase, aldehyde dehydrogenase, or combinations thereof.

Claim 16 (Withdrawn): The method of claim 1 wherein the bioengineering objective is overproduction of 1,3-propanediol and at least one candidate is for gene deletion and comprising genes coding for the enzymes fructose- 1,6-bisphosphatase, fructose- 1,6-bisphosphatase aldolase, phosphoglycerate kinase, glyceraldehyde-3-phosphate dehydrogenase, triosphosphate isomerase, glucose 6-phosphate-1-dehydrogenase, 6-phosphogluconolactonase, deoxyribose-phosphate aldolase, aldehyde dehydrogenase, or combinations thereof.

Claim 17 (Withdrawn): The method of claim 1 wherein the bioengineering objective is overproduction of succinate and at least one candidate is for gene deletion and comprising genes coding for the enzymes pyruvate formate lyase, acetaldehyde dehydrogenase, pyruvate kinase, FOF1-ATPase, NADH dehydrogenase I, fumarase, D-Lactate dehydrogenase, pyridine nucleotide transhydrogenase, phosphotransacetylase, acetate kinase, phosphotransferase, or combinations thereof.

Claim 18 (Currently amended): The method of claim 1 wherein the bioengineering objective function is overproduction of lactate and at least one candidate is for gene deletion and comprising genes coding for the enzymes phosphotransacetylase, acetate kinase, phosphofructokinase, fructose-4 1,6-bisphosphatase aldolase, triosphosphate isomerase, acetaldehyde dehydrogenase, glucokinase, or combinations thereof.

Claim 19 (Currently amended): A computer-based method for determining candidates for gene deletions and additions using a model of a metabolic network associated with an organism,

the model comprising a plurality of metabolic reactions defining metabolite relationships, the method comprising:

inputting at least one bioengineering objective function;

receiving as input as least one cellular objective function;

forming [[an]]a linear optimization problem that quantifies the at least one cellular objective function as an aggregate reaction flux and couples the at least one cellular objective function with the at least one bioengineering objective;

solving the linear optimization problem to yield at least one candidate; and outputting the at least one candidate.

Claim 20 (Currently amended):      The method of claim 1 wherein the cellular objective function comprises maximizing a growth rate.